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Nitrogen status of cotton subjected to two short term periods of waterlogging of varying severity using a sloping plot water-table facility

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Summary Cotton is reported to be susceptible to waterlogging, and there is evidence that some of the symptoms shown by waterlogged plants are due to impaired uptake of nitrogen. To investigate this for cotton, the nitrogen nutrition of a field-grown crop was monitored when the plants were subjected to two short term periods of waterlogging of varying severity using a sloping plot water-table facility. Growth of severely waterlogged cotton decreased after 4 days in the first and second floodings, and these plants were wilted by the end of the first flooding but not the second. Waterlogging resulted in decreased concentrations of total-N and especially NO_3^--N in the petiole and lamina of the youngest fully-expanded leaf. Uptake of N by waterlogged plants occurred, but was not as great as for well-aerated plants. The nitrate reductase activity of leaves was much lower in waterlogged plants. Stumps of detopped waterlogged plants did not exude xylem sap at the end of the first flooding, suggesting impaired solute uptake due to damaged roots. However, xylem exudate was obtained from stumps of waterlogged plants at the end of the second flooding, indicating adaptive changes to the root systems of these plants. Although cotton is reported to reduce little NO_3^--N in its roots, analysis of xylem exudate showed that about half of the N exported by roots was as amino compounds. The concentration of amino compounds in xylem exudate from severely waterlogged plants was higher than in well-aerated plants. It was concluded that the growth reduction in waterlogged cotton was due partly to induced N-deficiency.

Introduction

Most cotton crops in Australia are grown under flood irrigation on fine-textured heavy soils with low saturated hydraulic conductivity[14]. This may result in periods when the crop is waterlogged, especially if rainfall occurs soon after irrigation. Brief waterlogging can damage roots[24], and greatly reduce yields[9,39]. Characteristic symptoms of waterlogging are growth cessation, leaf epinasty and senescence, and the formation of adventitious roots[17,20,21]. However, the physiological mechanisms which bring about these symptoms are poorly understood. Physiological events which may occur during waterlogging include the accumulation of proline in shoots[1], the

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production of abscissic acid and ethylene[34], decreased cytokinin synthesis in roots, and impaired uptake and metabolism of key nutrients such as nitrogen and phosphorus[6,7,22,23,37,38].

Cotton is susceptible to waterlogging or excessive irrigation[5,13,14,16], and its growth and tissue levels of N are reduced by a high static water-table[26]. This paper reports on the nitrogen nutrition of cotton subjected to two short term periods of waterlogging of varying severity using a sloping plot water-table facility.

Materials and methods

Water-table facility

The sloping plot facility used to obtain a variable water-table depth, and the establishment of the cotton crop have been described previously[30]. Briefly, the facility was a sloping plot 45 m long by 5 m wide constructed on Hanwood Clay Loam Soil[35] at the CSIRO Centre for Irrigation Research, Griffith, central western N.S.W., in which a gradient of water-table depth was established by excavating a trench to a slope of 1.78%. The trench was lined with impervious fabric and the original soil was repacked giving 0.2 m of top soil and 0.4 m of subsoil with a uniform rooting depth of 0.6 m. The repacked soil overlay a gravel layer 0.15 m thick which facilitated movement of water during flooding or draining. Water entered the sloping plot through a slotted PVC pipe embedded in the gravel layer during flooding, and a float valve maintained the water level. At the end of the flooding, water was drained from the sloping plot by pumping from the gravel layer.

Six positions were selected for plant sampling at 3, 9, 15, 21, 27 and 43 m along the trench to provide water-table depths of + 0.04, −0.05, −0.17, −0.27, −0.38 and −0.60 m with respect to the soil surface. Plants growing where the water-table was 0.60 m below the surface were regarded as unstressed, and those growing where the water-table was 0.04 m above the soil were regarded as the plants stressed most severely. The first flooding lasted 8 days from 17 to 24 January, 1983, when the plants were 0.6 m high with a closed canopy and had just started flowering. The second flooding lasted 16 days from 7 to 23 March, 1983 when the plants were about 1 m high and had developing bolls.

Plant material

Cotton (*Gossypium hirsutum* L. cv. Deltapine 61) was sown on 26 October, 1982 in rows 0.25 m apart. Prior to sowing, fertilizer was incorporated at 130 and 100 kg ha^{-1} for N (urea) and P (P_2O_5), respectively. Seedlings were thinned to 19 plants m^{-2} two weeks after emergence. Weeds were controlled by herbicides or hand hoeing, and insect pests and mites were controlled chemically. Plants in the sloping plot were kept well watered by irrigation with an overhead sprinkler system.

A control area 45 m long and 5 m wide was established adjacent to the water-table facility. The soil and plants in this area were treated in the same manner as in the sloping, plot, except that half of the control area received no nitrogen to assess crop response to the applied N. The control area which received N is designated high-N control and that which received no N is designated low-N control. A top dressing of 75 kg N ha^{-1} as urea was applied to the sloping plot to assist plant recovery after the first flooding, and to the high-N control plants.

Harvest of plant material

The day before both floodings began and at the end of each period of waterlogging, 6 representative plants were cut at soil level from a zone 1.5 m either side of each water-table depth for tissue analysis. Leaf laminae were separated from stems and petioles, and bolls were also kept separate at the end of the second flooding. To hasten maturity at the end of the growing season, plants were defoliated with a dessicant, and yield was determined on

25 May 1983 for each water-table depth and the controls from 10 representative areas of 1 m^2. Mature bolls were separated into lint plus seeds and rest of boll.

The concentration of nitrate in the petiole of the youngest full-expanded leaf was used as an indicator of the N status of cotton[25]. Beginning the day before each flooding, two representative samples each of 12 youngest fully-expanded leaves were collected from the main axes of plants at each water-table depth along the sloping plot and the controls, and separated into laminae and petioles. These samples were taken daily during the first flooding, and at intervals of 2 or 3 days in the second. All tissue samples were collected between 11.00 and 12.00 h Australian Eastern Time to minimise possible diurnal effects.

Collection of root exudate

Root functioning was assessed by measuring the rate of xylem exudation and N content of xylem sap from stem stumps. At the end of the first flooding, and at the beginning and end of the second, three representative plants of similar size from each water-table depth and the controls were cut off 2 cm above soil level and xylem sap collected from 11.00 h to 13.00 h into plastic tubing fitted over the stumps. Sap from plants at each locality was transferred into pre-weighed plastic vials.

Analytical procedures

Plant parts were dried at 80°C and milled to pass a 20 mesh (0.84 mm) screen. Subsamples (250 mg) of plant material were digested by a Kjeldahl method with 0.5% Se as catalyst and salicylic acid to reduce nitrate-N (NO_3^--N)[8]. Concentrations of total-N were determined from the diluted digests with an autoanalyser using the indophenol blue method. Nitrate-N was extracted from 100 mg subsamples of plant tissue with 2*M* KCl, and determined on the autoanalyser[12]. Nitrate-N in xylem sap was determined by ion chromatography. Total amino-N in xylem sap was determined colorimetrically[41].

Assay of nitrate reductase

For assay of nitrate reductase, two samples each of 15 leaves were collected and placed onto ice at midday on the last day of the first flooding from plants at water-table depths of +0.04, −0.17 and −0.60 m, and transported to the laboratory within 15 minutes. An *in vivo* procedure was used, in which leaf laminae were finely chopped in a cold room at 3°C, and weighed subsamples transferred to flasks containing 12 ml of 30 m*M* KNO_3, 20 ml of 100 m*M* phosphate buffer (pH 7.5) and 0.4 ml of 0.13 *M* 1-propanol (1% v/v) which had been bubbled for 5 min with N_2 to remove air. One set of flasks had the KNO_3 replaced with distilled H_2O so that enzyme activity relied on nitrate present in the leaf tissue. The flasks were evacuated three times on ice to facilitate infiltration of the incubation mixture into leaf tissue, and the vacuum released under N_2. The flasks were stoppered with suba seals and transferred to a water bath at 30°C. At intervals of 10 min, 1 ml aliquots were withdrawn with a syringe and assayed for NO_2^--N[19]. Each value reported is the mean of two assays. The remainder of the leaf blade material and all petioles were assayed for total-N and NO_3^--N as described above.

Results

Plant growth

Waterlogged plants did not change in appearance during the initial four days of the first flooding, but on day 5 leaves of plants from the four wettest treatments had a greyish-green appearance. By day 7 some plants from these treatments had wilted, and by day 8 about 80% of these plants had wilted. Leaf growth was the same for all treatments until 4 days after the flooding began, then decreased in the four wettest treatments[30]. Watterlogged plants had recovered by the time the second

Table 1. Cotton yield data at final harvest. Values are means of at least 10 representative samplings of 1 m^2 at each water-table depth of the sloping plot. Values followed by the same letter do not differ at $P = 0.05$

Water-table depth (m)	Mean no. bolls $plant^{-1}$	Mean wt bolls $plant^{-1}$ (g)	Mean wt (lint + seed) $boll^{-1}$ (g)	Mean wt boll residue (g)
+ 0.04	5.3 a	25.4 b	3.46 c	1.34 c
− 0.05	6.3 b	30.9 c	3.62 c	1.29 b
− 0.17	6.5 b	32.2 c	3.72 c	1.24 b
− 0.27	6.3 b	29.9 c	3.60 c	1.11 a
− 0.38	5.9 b	23.9 ab	2.81 a	1.25 b
− 0.60	6.1 b	25.0 b	2.76 a	1.32 bc
HNC	7.1 c	27.2 bc	2.65 a	1.16 a
LNC	5.3 a	22.0 a	3.00 b	1.17 a

HNC: high-N control.
LNC: low-N control.

Table 2. Effect of first flooding on concentrations of total-N and nitrate-N in the lamina and petiole of the youngest fully expanded leaf of cotton. Values in parentheses are concentrations of N for plants from the driest treatment of the sloping plot expressed as mg g^{-1} dry weight

	Leaves		Petioles	
Water-table depth (m)	Concn. as % of value for water-table of − 0.6 m	% change in N concn. during flooding	Concn. as % of value for water-table of − 0.6 m	% change in N concn. during flooding
Total-N				
+ 0.04	69.0	− 16.7	74.3	− 53.1
− 0.05	68.2	− 22.5	67.5	− 44.4
− 0.17	73.3	− 17.6	70.6	− 48.2
− 0.27	77.2	− 16.3	79.6	− 41.9
− 0.38	92.4	− 10.7	97.5	− 37.0
− 0.60	100.0 (34.7)	+ 12.5	100.0 (16.2)	− 31.1
HNC	95.8	+ 11.3	99.4	− 34.3
LNC	61.1	0	51.1	− 29.4
Nitrate-N				
+ 0.04	36.6	− 58.9	60.4	− 76.3
− 0.05	67.0	− 1.2	55.2	− 70.3
− 0.17	63.4	+ 12.2	53.5	− 72.7
− 0.27	83.3	+ 17.8	66.4	− 65.1
− 0.38	65.4	+ 17.3	82.8	− 66.8
− 0.60	100.0 (0.25)	+ 11.8	100.0 (4.75)	− 53.0
HNC	74.0	− 1.1	100.3	− 64.0
LNC	17.4	− 45.6	13.9	− 95.7

HNC: high-N control.
LNC: low-N control.

flooding began and appeared uniform, but those from the four wettest treatments had slightly smaller upper leaves than the high-N control plants or those where water-table depths were −0.38 to −0.60 m. There were no signs of plant water stress (wilting) during the second flooding, although leaf growth rates decreased for plants from the four wettest treatments[30].

Yield data of mature plants are shown in Table 1. The number of bolls per plant was lowest at the most waterlogged end of the sloping plot and the low-N control, and highest for the high-N control. The mean number of bolls per plant was highest at water-table depths of −0.05, −0.17 and −0.27 m, as was the mean weight per boll. Plants from these treatments also had the highest yield of lint and seed per boll. However, bolls from the −0.38 and −0.60 m water-table depths and the high-N control were not quite mature at harvest, and probably would have gained further dry matter.

Concentrations of nitrogen in leaves

First flooding. Concentrations of total-N in the youngest fully-expanded leaf laminae (hereafter called leaves) decreased during the first flooding at all but the driest treatment and the high N control (Table 2). The average concentration of N in leaves of plants from the sloping plot at the beginning of the flooding was 3.1%, and by the end of the flooding this had fallen to 2.5% in leaves of plants from water-table depths of +0.04, −0.05, −0.17 and −0.27 m, but had risen to 3.3% in leaves of plants from the two driest treatments.

The situation for petiole N was similar to that for leaves, except for decreases in N concentrations in petioles from the two driest treatments

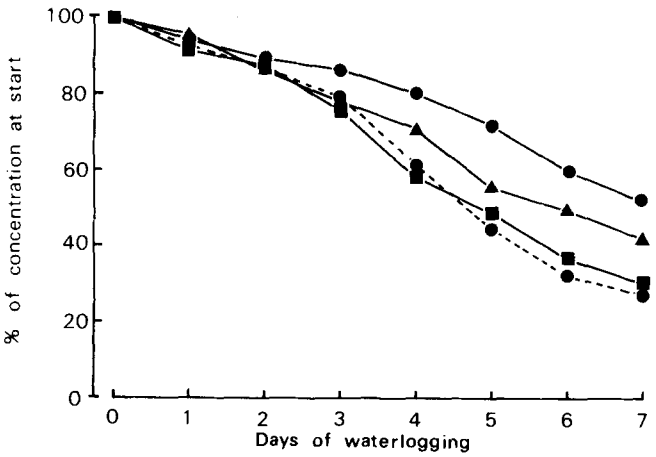

Fig. 1. Time courses of decline in concentrations of nitrate-N in petioles of youngest fully-expanded leaves of cotton subjected to short term waterlogging at selected positions along the sloping plot during first flooding. *Symbols* for water-table depths are as follows: ●----●, +0.04 m; ■——■, −0.17 m; ▲——▲, −0.27 m; ●——●, −0.60 m.

and the high-N control during the flooding; decreases in petiole N were greatest in plants from the wettest treatment.

The pattern of changes in concentrations of NO_3^--N in leaves and petioles was similar to that for total-N, although petiole NO_3^--N levels decreased to a greater extent than did concentrations of total-N. The major decrease in petiole NO_3^--N of waterlogged plants occurred three to four days after flooding began, whereas the decline in petiole NO_3^--N in plants from the two driest treatments and the high-N control was more gradual (Fig. 1). The average concentration of NO_3^--N in petioles of all plants from the sloping plot at the beginning of the flooding was 1.04% (dry wt basis) and this had fallen to 0.28% for plants from the four wettest treatments by the end of the flooding. These plants were at the threshold of N deficiency, as the value of 0.28% is close to the critical level (0.20%) for petiole NO_3^--N in cotton[11].

Second flooding. Although there were no symptoms of plant water

Table 3. Effect of second flooding on concentrations of total-N and nitrate-N in the lamina and petiole of the youngest fully expanded leaf of cotton. Values in parentheses are concentrations of N for plants from the driest treatment of the sloping plot expressed as mg g^{-1} dry weight.

	Leaves		Petioles	
Water-table depth (m)	Concn. as % of value for water-table of – 0.6 m	% change in N concn. during flooding	Concn. as % of value for water-table of – 0.6 m	% change in N concn. during flooding
Total-N				
+ 0.04	71.4	– 25.9	59.4	– 44.8
– 0.05	78.2	– 23.1	67.3	– 36.3
– 0.17	91.9	– 5.3	80.0	– 26.8
– 0.27	97.3	– 6.6	90.7	– 26.3
– 0.38	92.3	– 12.3	84.0	– 23.6
– 0.60	100.0 (37.1)	+ 4.2	100.0 (15.0)	+ 7.9
HNC	102.3	+ 4.6	83.3	– 16.9
LNC	68.5	+ 6.1	58.2	+ 27.3
Nitrate-N				
+ 0.04	38.8	– 51.1	3.4	– 98.0
– 0.05	44.1	– 67.2	10.0	– 94.4
– 0.17	49.7	– 52.7	51.3	– 72.6
– 0.27	59.2	– 70.6	72.3	– 74.5
– 0.38	68.7	– 39.7	60.6	– 73.7
– 0.60	100.0 (0.18)	– 8.2	100.0 (2.51)	– 19.5
HNC	75.4	– 29.0	87.0	– 25.9
LNC	41.9	+ 1.4	3.2	+ 5.2

HNC: high-N control.
LNC: low-N control.

stress in the second flooding, concentrations of total-N decreased in the youngest fully-expanded leaves and petioles of plants from all but the driest treatment of the sloping plot, and concentrations of NO_3^--N decreased in leaves and petioles of plants from all treatments, except for the low-N controls (Table 3). As with the first flooding, the decreases in leaf total-N and NO_3^--N were greatest in severely waterlogged plants. Nitrate concentrations in petioles of plants from the two wettest treatments and the low-N control were well below sufficiency levels[11], and indicate that these plants were N-stressed, despite the lack of visual symptoms of N deficiency.

Concentrations of nitrogen in whole plant tops

There was a significant decrease in concentrations of total-N in leaves and stems of plants from all treatments during the first flooding, except for the driest treatment and the high-N control (Table 4). Concentrations of total-N in leaves and stems of plants from the low-N control were significantly lower than those recorded for plants from the sloping plot or the high-N control. Levels of NO_3^--N decreased in plant tops from all treatments (including the controls) during the first flooding; however, the magnitude of the decrease in NO_3^--N was greater in severely waterlogged plants.

In the second flooding the pattern for leaf and stem total-N and NO_3^--N was similar to that of the first (Table 4), with a few exceptions. There were no changes in concentrations of NO_3^--N in plants from the low-N control; these were negligible even before the flooding commenced.

Amounts of nitrogen in plant tops and in mature bolls

Plants that were not waterlogged made the largest gains in N during the first flooding (Table 5). Plants from the wettest treatment lost N, and this was probably due to leakage of N solutes from damaged roots. All plants gained N during the second flooding, but the gains were proportionally much less than during the first flooding; plants from the wettest treatments acquired the least amount of N. At the end of the second flooding, bolls had accumulated between 31–44% of the total above-ground plant N, and this, plus the relatively small gain in N by plant tops, indicates that internal redistribution of N had occurred.

Concentrations of N in mature boll residues were lowest for plants from the three wettest treatments in the sloping plot (Table 6), but there were few differences in concentrations of N in lint + seed. Bolls from the driest treatment of the sloping plot had the greatest quality of

Table 4. Concentrations of total-N and nitrate-N in whole cotton plants subjected to two short-term periods of waterlogging. All values expressed as mg g^{-1} dry weight. Petioles included with stem

	Total-N				Nitrate-N			
	Leaves		Stem		Leaves		Stem	
Water-table depth (m)	Start	Finish	Start	Finish	Start	Finish	Start	Finish
First flooding								
+ 0.04	32.9 *	27.4	20.0 **	11.2	1.04 **	0.19	3.21 **	0.82
− 0.05	33.5 *	28.7	16.8 *	13.7	1.52 **	0.40	3.15 **	1.34
− 0.17	37.9 *	33.0	18.3 ns	15.0	2.43 **	0.78	3.34 **	1.81
− 0.27	37.2 *	30.1	18.9 *	13.2	1.82 **	0.25	4.08 **	1.11
− 0.38	40.2 *	35.9	22.7 *	15.8	1.61 **	0.31	5.12 **	1.65
− 0.60	35.6 ns	36.6	18.1 ns	16.3	1.84 **	0.59	4.51 *	2.49
HNC	37.4 ns	34.6	17.8 ns	15.9	3.18 **	0.26	5.22 **	2.30
LNC	24.2 *	20.8	12.4 *	9.5	1.90 **	0.19	2.75 *	1.92
LSD ($P = 0.05$)	4.5	3.7	2.9	2.5	0.92	0.14	0.81	0.33
Second flooding								
+ 0.04	34.2 *	27.4	12.8 *	7.2	0.52 **	0.09	1.05 **	0.26
− 0.05	28.7 ns	26.3	15.1 *	10.0	0.45 *	0.25	1.49 **	0.68
− 0.17	32.9 *	28.2	14.0 **	8.3	0.60 **	0.25	1.12 *	0.99
− 0.27	32.3 *	28.1	16.1 *	10.8	0.70 **	0.17	1.50 **	0.88
− 0.38	32. 6 ns	30.7	17.2 **	11.6	1.92 **	0.29	2.13 **	1.05
− 0.60	31.5 ns	32.8	16.2 *	12.8	1.31 *	1.01	2.28 **	1.46
HNC	34.3 *	29.5	15.1 *	10.7	0.61 **	0.16	1.23 *	0.88
LNC	18.6 ns	17.6	6.4 *	5.2	0.05 ns	0.05	0.07 ns	0.08
LSD ($P = 0.05$)	4.9	4.2	1.3	1.7	0.11	0.18	0.30	0.28

HNC: high-N control.
LNC: low-N control.
* $P = 0.05$.
** $P = 0.01$.
ns – not significant

N, and those from the low-N control the least. The percentage of N in mature bolls in the lint + seed fraction ranged from 42 (high-N control) to 55 (water-table depth of − 0.05 m).

Exudation from roots of detopped plants

Stumps of de-topped plants from the four wettest treatments did not exude xylem sap after the first flooding, suggesting damage to their root systems (Table 7). However, xylem exudate was obtained from plants halfway between the − 0.27 and − 0.38 m water-table depths, and the exudation rate increased noticeably as the water-table became

Table 5. Amounts of total-N in whole cotton plants subjected to two short term periods waterlogging. All values expressed as mg N plant part^{-1}. Petioles included with stem

	Start of flooding			Finish of flooding				
Water-table depth (m)	Leaves	Stems	Plant top	Leaves	Stems	Plant top	Bolls*	Gain in N plant top (%)
First flooding								
+ 0.04	0.39	0.26	0.65	0.41	0.17	0.58		– 10.7
– 0.05	0.52	0.21	0.73	0.56	0.25	0.81	–	11.0
– 0.17	0.58	0.25	0.83	0.71	0.29	1.00	–	20.5
– 0.27	0.45	0.21	0.66	0.64	0.26	0.90		36.4
– 0.38	0.58	0.30	0.88	0.95	0.33	1.28	–	45.5
– 0.60	0.63	0.25	0.88	0.97	0.38	1.35		53.4
HNC	0.67	0.29	0.96	0.89	0.50	1.39	–	44.8
LNC	0.28	0.13	0.41	0.35	0.16	0.51	–	24.3
Second flooding								
+ 0.04	0.82	0.78	1.60	0.59	0.36	0.73	1.68	5.0
– 0.05	0.69	0.93	1.62	0.55	0.41	0.75	1.71	5.6
– 0.17	0.74	0.85	1.59	0.67	0.33	0.68	1.68	5.7
– 0.27	0.72	1.03	1.75	0.51	0.62	0.73	1.86	6.3
– 0.38	0.76	1.09	1.85	0.66	0.76	0.65	2.07	11.9
– 0.60	0.82	1.06	1.88	0.77	0.67	0.79	2.23	18.6
HNC	0.97	1.82	0.75	0.75	0.68	0.64	2.07	13.7
LNC	0.22	0.24	0.46	0.17	0.17	0.18	0.52	13.0

HNC: high-N control.
LNC: low-N control.
* Bolls present only at end of second flooding.

lower. The exudation rate from high-N control plants was double that of plants from the driest treatment of the sloping plot.

Before the second flooding, xylem sap was obtained from plants at all localities of the sloping plot and the controls. The rate of exudation was lowest for plants from the localities of the sloping plot which had been wettest during the first flooding, suggesting that these plants had not recovered fully from the first flooding (Table 7). The exudation rate from low-N control plants was much less than that of plants from any water-table depth of the sloping plot or the high-N control.

The pattern was similar at the end of the second flooding, although there was a decline in the exudation rate of plants from all treatments of the sloping plot and the controls (Table 7). Differences in the exudation rates of plants from the driest treatment and the high-N controls were not as great as in the first flooding, suggesting that the plants responded differently as they aged.

Nitrogen in xylem sap

The cotton plants used in this experiment transported about equal

Table 6. Concentrations and amounts of total-N in the boll residue and lint plus seed at maturity of cotton plants subjected to short term waterlogging. Values followed by the same letter do not differ at $P = 0.05$

	Concentration of total N in:		Amount of N in:		
Water-table depth (m)	Boll residue (mg g^{-1} dry wt)	Lint + seed (mg g^{-1} dry wt)	Boll residue (mg $boll^{-1}$)	Lint + seed (mg $boll^{-1}$)	Total amount of N in mature boll (mg $boll^{-1}$)
+ 0.04	13.5 ab	20.1 b	18.1 c	69.5 b	87.6 c
− 0.05	11.7 a	19.3 ab	15.1 b	69.9 b	85.0 c
− 0.17	12.9 a	20.3 b	16.0 b	75.5 c	91.5 d
− 0.27	16.5 b	19.7 b	18.3 c	70.9 b	89.2 cd
− 0.38	16.5 b	19.2 ab	20.6 d	55.4 a	76.0 b
− 0.60	17.1 b	19.6 b	22.6 e	80.0 c	102.6 e
HNC	19.8 c	20.7 b	23.0 e	54.9 a	77.9 b
LNC	12.5 a	18.7 a	14.6 a	56.1 a	70.7 a

HNC: high-N control.
LNC: low-N control.

Table 7. Rates of xylem sap bleeding from detopped cotton plants subjected to short term waterlogging. Values are means of three plants

	Volume of sap h^{-1} $plant^{-1}$ (μl)		
Water-table depth (m)	End flooding 1 (24/1/83)	Start flooding 2 (4/3/83)	End flooding 2 (22/3/83)
+ 0.04	0	273 **	6
− 0.05	0	422 **	62
− 0.17	0	476 **	113
− 0.27	0	572 **	124
− 0.33	116	–	–
− 0.38	199	536 **	214
− 0.52	237	–	–
− 0.60	1642	612 **	284
HNC	2973	641 **	372
LNC	–	157 **	30

HNC: high-N control.
LNC: low-N control.
For second flooding: **, $P = 0.01$.

amounts of NO_3^--N and amino-N in the xylem sap (Table 8). At the end of the first flooding, concentrations of NO_3^--N were similar for plants of all treatments from which sap was obtained, and concentrations of amino-N were also similar for each treatment. However, fluxes of NO_3^--N and amino-N were much greater for the driest treatment than for the others because of the higher exudation rate, and there was a further increase in the flux of N between the driest treatment and the high-N control.

Table 8. Concentrations and fluxes of nitrogenous solutes in xylem sap bleeding from detopped cotton plants subjected to waterlogging. Values are means of three plants from each treatment

Water-table depth (m)	End flooding 1				Start flooding 2				End flooding 2			
	Nitrate-N		Amino-N		Nitrate-N		Amino-N		Nitrate-N		Amino-N	
	Concn. (μg ml^{-1})	Flux (μg ml^{-1} h^{-1})	Concn. (μmol ml^{-1})	Flux (μmol ml^{-1} h^{-1})	Concn. (μg ml^{-1})	Flux (μg ml^{-1} h^{-1})	Concn. (μmol ml^{-1})	Flux (μmol ml^{-1} h^{-1})	Concn. (μg ml^{-1})	Flux (μg ml^{-1} h^{-1})	Concn. (μmol ml^{-1})	Flux (μmol ml^{-1} h^{-1})
+ 0.04	–	–	–	–	32.7	8.9	3.4	0.9	27.1	0.2	6.6	0.04
– 0.05	–	–	–	–	42.1	17.8	2.9	1.2	29.4	1.8	6.5	0.40
– 0.17	–	–	–	–	49.1	23.4	2.8	1.3	32.1	3.6	5.4	0.61
– 0.27	–	–	–	–	37.4	21.4	0.9	0.5	31.7	3.9	8.9	1.10
– 0.33	51.4	6.0	9.0	1.0	–	–	–	–	–	–	–	–
– 0.38	59.5	11.9	7.2	1.4	59.1	31.7	1.3	0.7	39.9	8.6	4.4	0.93
– 0.52	44.2	10.5	6.7	1.6	–	–	–	–	–	–	–	–
– 0.60	48.0	78.8	4.1	6.7	46.7	28.6	1.1	0.7	37.7	10.7	4.7	1.34
HNC	47.2	140.3	6.0	17.8	44.8	28.7	4.2	2.7	38.7	14.4	6.2	2.31
LNC	–	–	–	–	22.5	3.5	1.6	0.3	15.0	0.4	1.5	0.05

HNC: high-N control.
LNC: low-N control.

Table 9. Nitrate reductase activity and nitrogen concentrations in leaves of cotton plants at the end of the first waterlogging. Leaf material was incubated *in vivo*, with and without nitrate added to the incubation mixture. Values are means of two separate assays

			Concentration (mg g^{-1} dry wt) of:			
	Nitrate reductase activity (μ mols NO_3 g fresh wt $leaves^{-1}$ h^{-1})		Total-N		Nitrate-N	
Water-table depth (m)	+ NO_3	− NO_3	Petioles	Leaves	Petioles	Leaves
+ 0.04	0.52	0.14	12.0	21.0	2.9	0.1
− 0.27	1.46	0.30	12.9	25.4	3.2	0.3
− 0.60	2.07	0.69	16.2	32.0	4.8	0.5

Before the start of the second flooding, concentrations of NO_3^--N were similar in all treatments, with the exception of low values for plants which had been ponded in the first flooding and those of the low-N control (Table 8). Amino-N was more concentrated in exudate of plants from the section of the sloping plot which had been waterlogged severely in the first flooding. At the end of the second flooding, concentrations of NO_3^--N in xylem sap had declined in all plants from the sloping plot and the controls, and fluxes of NO_3^--N were lowest in the zone of worst waterlogging (water-table depths +0.04 to −0.27 m). Concentrations of amino-N were highest in the xylem sap of plants from the wettest treatments, although fluxes of amino-N into these plants were low because of low rates of exudation.

Nitrate reductase activity

Nitrate reductase activity (NRA) was estimated only at the end of the first flooding. In the absence of added nitrate in the incubation mixture, NRA increased as the depth of the water-table of the sloping plot increased (Table 9). The values for NRA when nitrate was added, thus relieving any substrate deficiency, indicate that the enzyme was present or active to a much lesser extent in leaves of severely waterlogged plants. The NRA levels paralleled the values for concentrations of total-N and NO_3^--N in leaves and petioles (Table 9).

Discussion

It is clear that the two periods of waterlogging brought about a reduction in the growth of the cotton plants, and affected N uptake and assimilation. However, the responses of cotton were less dramatic than expected, in view of its reported susceptibility to waterlogging[5,13,14]. The lack of water stress symptoms at the end of the second flooding after 2 weeks of waterlogging[30] suggests that the plants

Table 10. Percentage of roots above the water-table at each water-table depth at the beginning of the first and second floodings. See reference 30 for details of technique used to observe root distribution in this study

Water-table depth (m)	% of roots above water-table	
	First flooding	Second flooding
+ 0.04	0	0
− 0.05	0	10
− 0.17	25	55
− 0.27	40	70
− 0.38	62	80
− 0.60	100	100

had adapted during and after the first flooding. In addition, the first flooding brought about a change in root distribution, as a greater proportion of the roots occurred above the water-table at the start of the second flooding (Table 10).

A comparison of the data for the low-N and high-N controls shows that the cotton plants responded to N fertilizer. Concentrations of total-N and NO_3^--N in leaves and petioles of low-N control plants were in the deficient range[11], and by the end of each flooding NO_3^--N concentrations in petioles of the severely waterlogged plants also indicated N deficiency.

During both floodings, concentrations of total-N and NO_3^--N declined in petioles and leaves of plants at the well-aerated end of the sloping plot and from the high-N control. This has been attributed partly to aging effects, as it has been shown[36] that N concentrations decline with age in vegetative organs of cotton. It is also probably due to depletion of N in the rooting zone. Changes in tissue concentrations of total-N in low-N control plants due to aging and depletion of soil N reserves were not as pronounced as in plants from the high-N control, as found in earlier work[36] with N-stressed cotton.

An unusual feature of this study was that reduced growth of waterlogged plants in the second flooding was not associated with decreased leaf water potentials or reduced transpiration rates[30]; however, uptake of N (this paper) and other nutrients (Hocking *et al.*, unpubl. data) decreased. This suggests that water uptake due to transpiration is mainly a passive process not requiring O_2, and which continues despite decreased active uptake of nutrients by waterlogged roots.

It is clear that there was a rapid decline in tissue NO_3^--N after 3 days in the waterlogged cotton plants, which coincided with the onset of reduced growth of leaves[30]. However, the reduced growth cannot be attributed entirely to deficiency of nitrogen, as concentrations

of P and K also declined (Hocking *et al.* unpubl. data). Decreased concentrations of N, P, K and Ca have been reported in other studies of waterlogged crop plants[2,7,18,32,33,37], including cotton[26]. We conclude that root uptake of N and other elements is impaired before growth is reduced in waterlogged cotton, and this may result in the crop becoming deficient in several nutrients.

Reductions in exudation rates have been reported for herbaceous species when their root systems were subjected to anaerobic conditions[20,21] and the impaired capacity of waterlogged cotton plants to exude sap is likely to result from reduced uptake, transport and xylem loading of ions by roots. Uptake of NO_3^--N has been reported to cease when roots are waterlogged[37,40], but the effect of flooding on NO_3^--N uptake was not so clear cut in this experiment. For example, NO_3^--N was present in xylem sap at the end of the second flooding despite mean partial pressures of soil oxygen of 3 to 6 kPa in the soil solution. Plants in the wettest treatment lost N from their shoots during the first flooding (Table 5), and this was probably due to leakage of nitrogenous solutes from damaged roots.

Cytokinin synthesis in roots is impaired by waterlogging[3,4], and N deficiency appears to bring about a slowing of growth partly because of reduced export of cytokinins from roots[15]. In particular, NO_3^--N is linked with the synthesis of cytokinins[14], and thus decreased growth of waterlogged cotton in this experiment may have been due partly to reduced levels of cytokinins synthesized and exported from roots because of their poor NO_3^--N status.

Roots of cotton reduce only sufficient NO_3^--N for their requirements, and export the rest to the shoot[10,28,29]. However, in this experiment cotton roots exported about equal quantities of N as reduced-N (amino compounds) and NO_3^--N in xylem sap to the shoot, and there was a trend for a greater proportion of reduced-N with increased degree of waterlogging. The presence of large quantities of reduced-N in xylem sap could be due to ammonium uptake from the soil, although soil NH_4^+ values were low and variable[31]. It is likely that the higher concentrations of amino-N in waterlogged plants were due to the hydrolysis of proteins in necrotic root tissue, and the subsequent release of amino acids into the xylem. They may also be due to increased resident time of NO_3^--N in waterlogged roots, as suggested by the low flux of xylem exudate from stumps of waterlogged plants, thus giving more time in which reduction of NO_3^--N could occur.

It has been suggested[27] that NRA of the youngest fully-expanded leaf of cotton is a more sensitive indicator of plant N status than NO_3^--N concentrations in petioles. NRA also appears to be a good indicator

of plant stress due to waterlogging. Although nitrate added to the incubation mixture increased NRA, it did not increase the activity of the enzyme more in waterlogged plants than in well-aerated plants suggesting that the enzyme was not present in an inactive form to a greater extent in waterlogged plants than in the well-aerated ones.

In conclusion, it is likely that the decreased N uptake of waterlogged cotton is only partly responsible for the observed reduction in vegetative growth, as decreased uptake of other nutrients such as P and K may also contribute to the growth reduction. In practical terms, a short period of waterlogging may result in N deficiency in cotton, especially if the crop was growing vigorously and the availability of soil N was only just adequate before the waterlogging.

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